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Taxonomic segregation of the Swallowtails of the genus *Graphium* (Lepidoptera: Papilionidae) of Kerala part of Western Ghats using morphological characters of external genitalia

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Abstract

Studies on the genitalia of four species of Papilionids belonging to the tribe Leptocercini were made. The structure of vinculum, uncus, valvae and phallus of the male genitalia and the bursa, ductus and ovipositor of the female were found to be useful in taxonomic segregation of these butterflies. This highlights the extreme practical importance of external genitalic structures in the identification of these butterflies and improves upon earlier characters for generic and specific determinations based mainly on the wing venation, size and shape of palpi, and frons.

Keywords: Taxonomy, Papilionidae, Lepidoptera, *Graphium*, Western Ghats

1. Introduction

The Western Ghats constitute a mountain range along the western side of India. It is acclaimed as World Heritage Site by UNESCO and is one of the world's eight "hottest hotspots" of biological diversity. Southern Western Ghats extending from the Agasthamalai to Palghat Gap has highest butterfly diversity with maximum Endemics. Thirty six species of butterflies are reported to be endemic to the Ghat and among the butterfly genera, the genus *Parantirrhoea* is exclusively endemic to this region [1].

Butterflies are classified into two superfamilies, Hesperioidea and Papilionoidea. Of which Hesperioidea has all the skippers, while Papilionoidea includes the rest, the true butterflies. Hesperioidea consists of a single family of Hesperidae (Skippers), whereas Papilionoidea has four families: Papilionidae (Swallowtails), Pieridae (Whites and yellows), Nymphalidae (Brush-footed butterflies) and Lycaenidae (Blues). Among various groups of butterflies the Papilionids are very important since they contain some of the largest and colourful butterflies.

Papilionidae, which is the smallest butterfly family in the world, has only 701 species representing just 4% of global butterfly diversity. In India, there are about 107 species of Papilionid butterflies, of which 19 species are present in Kerala [8]. The Papilionids show high degree of endemism compared to the members of other families. The family Papilionidae is classified into 4 Tribes – Troiidiini, Papilionini, Leptocercini and Teinopalpini.

Kerala harbours five species of butterflies which fall under the tribe Leptocercini and genus *Graphium*. They include *G. doson* C&R Felder (Common Jay) and *G. agamemnon* Linnaeus (Tailed Jay), *G. sarpedon* Linnaeus (Common Bluebottle) and *G. nomius* Esper (Spot Swordtail) and *G. antiphates* Fabricius (Five-bar Swordtail). These are very nervous, restless butterflies, settling seldom and only momentarily and their flight is very straight and dashing. Most of these are swift, powerful fliers and occur in all vegetation layers. The Jays and Bluebottles have black wings with green or blue spots and short tails. The Jays and Bluebottles are found in evergreen, wet semi-evergreen, moist deciduous as well as thick, riparian forests. Due to cultivation and availability of larval host plants, the Jays and Bluebottles are often noticed in urban areas also. They are very passionate of nectar and are found in mud puddling aggregation but the Tailed Jay feeds exclusively on nectar and never noticed in mud puddling groups. The butterflies gather mainly around the riparian areas in the forests and settle on wet soil for mud puddling. Swordtails have white wings with black bands with red and green spots and the hindwings have sharp, pointed sword-like tails. Spot Swordtails inhabit dry deciduous forests. Five-bar Swordtails are found in evergreen, wet semi- evergreen, moist deciduous as well as thick riparian forests. The

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are common during dry months.

The Swallowtail is one of about 15 target species in a new campaign of research and conservation of British butterflies currently being run by WWF (U.K.). They have great ecological roles as bioindicators, pollinators and as energy transferors. Some of them are major pests of agricultural crops as well as forest trees. *Graphium agamemnon* Linnaeus, which is feeds on soursop (*Annona muricata* Linnaeus) in Vietnam. Larvae of *G. agamemnon* were found to damage soursop trees throughout the year but damage was more common in the middle of the wet season, which lasts from May to October [13]. *G. agamemnon* is distributed in India, South-East Asia to Australia including the nearby Pacific islands, where it feeds primarily on custard apple (*Annona reticulata* Linnaeus) and soursop (*Annona muricata* Linnaeus) [1]. No major taxonomic revision on the Indian species has appeared since the pioneer work by Talbot [12]. The early publications on this group were based mainly on the wing venation, larval host plants and colour of wings for specific determinations. Kerala, with its diversified habitats suggests a fairly large number of Swallowtail butterflies. A revision of this group was thus obvious. The taxonomic segregation of four species viz., *G. sarpedon* Linnaeus (Common bluebottle), *G. doson* C&R Felder (Common Jay), *G. agamemnon* Linnaeus (Tailed Jay) and *G. nomius* Esper (Spot swordtail) have been described here.

2. Materials and Methods

Sampling of butterflies was done by conducting a field survey in representative habitats in northern, central and southern Kerala during 2010-2012. Altogether, 6 locations were selected for sampling. The areas covered included Nilambur (11°17'58"N 76°15'03"E), Peechi (10° 31' N, 76° 24' E), Vazhani (10° 36.75' N 76° 24.42' E), Athirappilly (10° 17'19" N, 76° 32'54"E), Vazhachal (10° 14' N, 76° 25' E) and Thenmala (8°57'0"N and 77°4'0"E). At each of these locations, sampling sites were selected based on previous faunal records. Each area could be visited only once. Generally, six males and 6 females were used for genitalia studies. However, when enough samples could not be collected especially in the case of some rare and endemic butterflies, material available in the samples was utilized. In such cases, the abdomen of the specimen was clipped off and used for dissection. For preparing slide mounts of the external genitalia, the lower part of the abdomen (from the 7th segment onwards) was removed using micro-needles and treated with 10% KOH by gently boiling in a water bath. After appropriate maceration in KOH, the boiling was stopped and the material was thoroughly washed. The external genitalia were then extracted from the abdomen by gently tearing the inter-segmental membranes from and around the organ by using fine forceps and micro needles. After careful cleaning, the genitalia were stained with acid fuschin dissolved in acetic acid and transferred to carbol- xylol for clearing (Carbolic acid and xylene 2:3) and mounted in Canada balsam [10].

3. Results and discussion

3.1 List of butterflies

Family: Papilionidae

Sub family: Papilioninae

Tribe: Leptocercini

Genus: *Graphium* Scopoli

Scopoli, 1777. *Intr. Hist. Nat.*, p 433.

3.1.1 *Graphium sarpedon* Linnaeus (Common Bluebottle)

(=*Zetides sarpedon*) Linnaeus, 1758. *Syst. Nat.*, X: 461).

D'Abrera, 1982, *Butterflies of the Oriental Region*, Part 1: 98

Wynter-Blyth, 1957. *Butterflies of the Indian Region*, Bombay Natural History Society, p. 401.

Kehimkar, I. (2008). *The Book of Indian Butterflies*. Bombay Natural History Society, 497 pp.

Material in collection: 4♂4♀., 21.x.11, Nadugani Ghat.

Wing span: 73.8 (± 2.10) mm.

Distribution: Sri Lanka, Myanmar, Nepal, Singapore, Bangladesh and India: From India, specifically recorded from Kashmir, Assam and south India (Vazhachal, Nadukani, Peechi, Thenmala and Parambikulam).

Host plants: *Alseodaphne semicarpifolia*, *Cinnamomum camphora*, *C. malabathrum*, *C. macrocarpum*, *Polyalthia longifolia*, *Persea macrantha*, *Litsea chinensis* and *Miliusa tomentosa*.

Status: It is a common butterfly occurring at all elevations both in forests and in agricultural lands.

Remarks: Recorded from Nilambur, Nadugani Ghat, Peechi,

3.1.2 *Graphium doson* C. & R. Felder (Common Jay)

(=*Zetides doson*) Felder, 1864. *Verh. zool.-bot. Ges.* 1864: 365.

D'Abrera, 1982, *Butterflies of the Oriental Region*, Part 1: 100.

Wynter-Blyth, 1957. *Butterflies of the Indian Region*, Bombay Natural History Society, p. 402.

Kehimkar, I. (2008). *The Book of Indian Butterflies*. Bombay Natural History Society, 497 pp.

Material in collection: 3♂3♀., 9.ii.11, Peechi.

Wing span: 75.3 (± 2.45) mm.

Distribution: Sri Lanka, Myanmar, Nepal, Bhutan and India: From India, specifically recorded from Bengal, Kumaon to Assam and S. India to Maharashtra.

Host plants: *Michelia champaca*, *Annona lawii* and *Magnolia grandiflora*, *Polyalthia longifolia*, *Miliusa tomentosa*, *Cinnamomum macrocarpum* and *C. Malabathrum*.

Status: Locally Common.

Remarks: Recorded from Peechi and Nilambur

3.1.3 *Graphium agamemnon* Linnaeus (Tailed Jay)

(=*Zetides agamemnon*) *Graphium agamemnon*. Linnaeus, 1758. *Syst. Nat.* X: 462.

D'Abrera, 1982. *Butterflies of the Oriental Region*, Part 1: 102.

Wynter-Blyth, 1957. *Butterflies of the Indian Region*, Bombay Natural History Society, p. 404.

Kehimkar, I. (2008). *The Book of Indian Butterflies*. Bombay Natural History Society, 497 pp.

Material in collection: 4♂3♀., 17. xii.10, Peechi; 1♂., 16.i.11, Nilambur.

Wing span: 76.5 (± 5.23) mm.

Distribution: Oriental Region, Myanmar, Sri Lanka and India. From India, specifically recorded from S. India to Gujarat (Kutch), Uttaranchal to Arunachal Pradesh as well as Andaman and Nicobar

Islands.

Host plants: *Polyalthia longifolia*, *Uvaria narum*, *Michelia champaca*, *Artabotrys hexapetalus*, *Polyalthia cerasoides*, *Annona reticulata*, *A. squamosa*, *A. muricata* and *Cinnamomum* spp.

Status: Common.

Remarks: Recorded from Nilambur, Nadukani Ghat, Peechi and Thenmala.

3.1.4 *Graphium nomius* Esper (Spot Swordtail)

(=*Pathysa nomius*) Esper 1785-98. *Die Schmetterlinge* (=the butterflies) Supplement. pt. i. P. 210, t.52, figure3.

D'Abrera, 1982. *Butterflies of the Oriental Region*, Part 1: 110.

Wynter-Blyth, 1957. *Butterflies of the Indian Region*, Bombay Natural History Society, p. 398.

Kehimkar, I. (2008). *The Book of Indian Butterflies*. Bombay Natural History Society, 497 pp.

Material in collection: 3♂3♀., 6.ii.13, Peechi; 1♂1♀., 1.iii.13, Peechi.

Wing span: 74.2 (± 9.71) mm.

Distribution: Sri Lanka, Myanmar, Nepal, Bhutan and India: Peninsular India to S. Bihar, Madhya Pradesh, Lucknow, Simla and Assam.

Host plants: *Saccopetalum tomentosum* and *Polyalthia longifolia*.

Status: Rare.

Remarks: In Kerala it has been recorded from moist deciduous forests of Peechi and Vazhani during the summer season.

3.2 Description of external genitalia

3.2.1 *Graphium sarpedon* Felder & Felder (Common Bluebottle) (Pl. 1, Fig. 1; Pl.2, Fig. 1)

Male genitalia: Uncus short, more or less finger-shaped with a broad proximal part and a narrow, slightly curved and blunt apical part. Socii long, cylindrical and apically blunt situated below the uncus. Tegumen and Vinculum with elongated, narrow arms. Valvae ovate, having a deep invagination at the apex near the cucullus. Sharply pointed edges of invaginated portion of valva. A pair of pointed processes near cucullus. Costa strongly arched in outline. Harpe comprising of a flat plate bearing a narrow lobe-like appendage at the distal end, towards the costal margin. Sacculus straight and not sclerotised. Saccus U-shaped. Phallus very long and narrow with the apex having a notch leaving a small spine. Proximal end swollen with uneven margin.

Female genitalia: Ovipositor lobes long, narrow and fringed with hairs. Posterior apophyses approximately double the length of anterior. Sinus vaginalis broad. Ostium, small with sclerotised walls. Ductus bursae short, broad and almost of uniform width. Corpus bursae more or less balloon-shaped. A short, tubular signum present.

3.2.2 *Graphium doson* Felder (Common Jay) (Pl. 1, Fig. 2; Pl. 2, Fig. 2)

Male genitalia: Uncus broad, rectangular, bearing two lobes on either side. Tegumen and Vinculum with long narrow arms. Valvae ovate, lobe-shaped; costa little raised, slightly convex in outline, cucullus straight and sclerotised. Harpe flattened lobe

bearing four prominent spines. One pair pointing towards the ventral margin, the second pair towards cucullus and the third pair towards the costal margin. Saccular margin slightly curved. Saccus U-shaped. Phallus long with sharply pointed apex. Vesica pouch-like occurring subapically. Proximal end with a broad segment.

Female genitalia: Ovipositor short with broad lobes. Anterior apophyses longer than the posterior. Sinus vaginalis broad. Ostium wide. Ductus bursae short and wide. Corpus bursae globular and striated. Signum composed of a long, basally broad, blunt, tubular process.

3.2.3 *Graphium agamemnon* Linnaeus (Tailed Jay) (Pl. 1, Fig. 3; Pl.2, Fig. 3)

Male genitalia: Uncus broad ligulate, with deeply invaginated in the middle forming two lateral lobes each bearing a tuft of hairs either side. Socii and Gnathos not distinct. Tegumen and Vinculum long, almost straight with lateral sclerotisation. Juxta roughly rectangular and indented laterally. Valvae broad, more or less round. Costa straight and cucullus curved bearing numerous denticles. Harpe expanded, sclerotised and distally bearing a short blunt lobe. Sacculus broad at the distal end and tapering proximally. Saccus well developed, with a long U-shaped blunt process. Phallus very long slightly curved in the middle with a sharply pointed apex. Vesica pouch-like occurring subapically. Proximal end with a broad segment.

Female genitalia: Ovipositor lobes elongated, swollen and fringed with small hairs. Apophyses of equal size. Sinus vaginalis laterally expanded, narrow and boat-shaped. Ostium wide. Ductus bursae medium sized and of uniform width. Corpus bursae more or less globular with concentric rings. Signum composed of a pouch-like sclerotised body and another triangular process.

3.2.4 *Graphium nomius* Esper (Spot Swordtail) (Pl. 1, Fig. 4; Pl.2, Fig. 4)

Male genitalia: Uncus broad, ligulate, with a shallow invagination in the middle to form two slightly pointed lateral lobes. Socii and Gnathos not distinct. Tegumen and Vinculum with long narrow arms. Valvae short, oval with the apex slightly drawn out, costa straight with setose and bearing sclerotised patches at cucullus. Harpe composed of two basally expanded and distally pointed sclerotised spine-like processes. Sacculus broadly globular in outline. Juxta prominent. Saccus U-shaped and well developed. Phallus very long with a blunt tip, highly setosed and strongly sclerotised.

Female genitalia: Ovipositor broad and triangular. Anterior apophyses slightly longer than the posterior which has lobed endings. Sinus vaginalis narrow and short. Ductus bursae medium length, basally swollen. Corpus bursae roughly globular with a marginal sclerotised patch. Signum composed of a small spine-like process.

3.3 Wing venation

Forewing consists of 12 veins with a large cell called discal cell, usually closed with a number veins radiating from it. Among the 12 veins, the first and last are arising from the base and others from the discal cell. On the forewing, vein 12 is known as Subcosta (Sc); veins 11-7 are the Radial veins which have five branches (R1-R5); Veins 6-4 are the Median veins which have three branches M1-M3; veins 3-2 are the Cubital veins, Cu1a and Cu1b and the last vein is the Anal vein, which has two branches viz., 1A and 2A (Plate 4). The second anal vein is short and third Anal vein is totally absent.

In the hind wing, the first radial vein is fused with Subcosta (Sc and R1 are fused), thus vein 8 is Sc+R1; The Radius is not divided further and the remaining one is termed Radial sector (7 th vein, Rs). Veins 6-4 are the Median veins, they have three branches (M1-M3). veins 3-2 are the Cubital veins- Cu1a and Cu1b. Only one Anal vein is present viz., 1A. There is a small spur near the base of 8 th vein, projecting towards the costa, is called Humeral vein.

3.4 Morphological similarities and dissimilarities of the external genitalia in the Tribe Leptocercini

Four species, *Graphium sarpedon*, *G. agamemnon*, *G. nomius* and *G. doson* belonging to this tribe have been studied. Uncus is distinct and well developed in all the four species. In *G. sarpedon*, it is finger-shaped with a broad proximal part and blunt apical part. In *G. agamemnon* and *G. nomius*, uncus is ligulate with a middle invagination to form lobes. It is broad and rectangular with lateral lobes in *G. doson*.

Valvae ovate with an invagination at the apex near cucullus in *G.*

sarpedon. In *G. doson*, it is lobate whereas in *G. agamemnon*, round. In *G. nomius*, it is short with the apex slightly drawn out giving a conical appearance. Harpe shows wide range of variations in shape. It is flat, plate-like having a narrow lobe-like appendage at the distal end in *G. sarpedon*. An expanded, sclerotised harpe bearing a distal short blunt lobe in *G. agamemnon*. Harpe comprises of two basally expanded and distally pointed sclerotised spine-like processes in *G. nomius*. In *G. doson*, harpe is flattened lobe-like bearing four prominent spines- one pair pointing towards the ventral margin, the second pair towards cucullus and the third pair towards the costal margin. The modifications of the phallus mostly involved its shape and size. It is long and narrow with an apical notch leaving a small spine in *G. sarpedon*. Long Phallus with sharply pointed apex and pouch-like vesica is situated sub-apically in *G. agamemnon* and *G. doson*. It is long with a blunt tip in *G. nomius*.

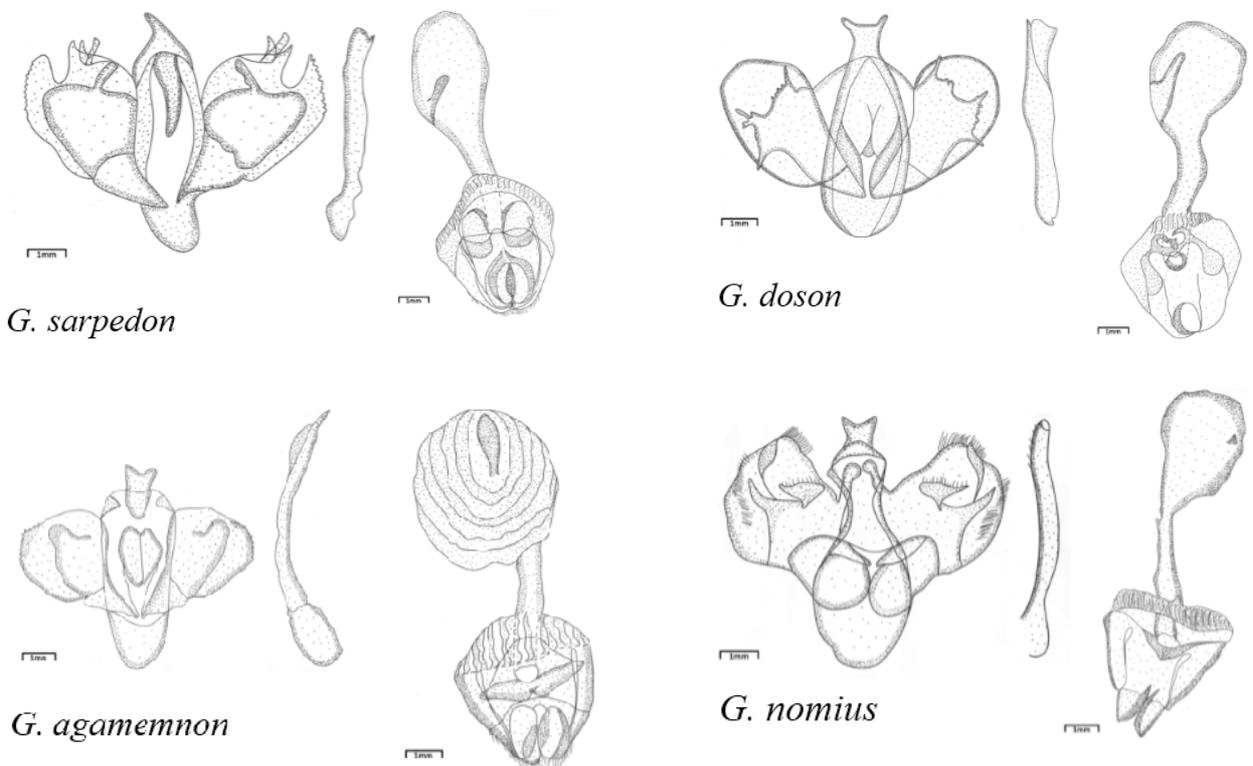


Plate 1: Illustrations of male and female external genitalia. Figs: 1. *G. sarpedon*; 2. *G. doson*; 3. *G. agamemnon*; 4. *G. nomius*

The members of the tribe Leptocercini showed great similarity in the structure and shape of the tegumen, vinculum and saccus. Tegumen and vinculum were elongated with narrow arms. Saccus was broadly oval in all four species.

The female genitalic characters of all the four species were studied and they showed a great similarity in structure. Ovipositor lobes were broad and triangular in *G. nomius*; elongated and swollen in *G. agamemnon*, *G. doson* and *G. sarpedon*. Ductus bursa was elongated in *G. nomius* whereas it was moderate in length and uniform in width in the remaining species. Corpus bursa was more or less globular in *G. agamemnon*; tubular or oval in shape in *G. sarpedon*, *G. nomius*, *G. doson*. Signum was prominent and tubular in *G.*

sarpedon, *G. agamemnon* and *G. doson* while short in *G. nomius*.

3.5 Key for the identification of species

1. Valvae notched and dentate; phallus elongated.....*G. sarpedon*
- Valvae not notched2
2. Harpe expanded lobe with irregularly dentate margins.....*G. doson*
- Harpe without irregular lobes.....3
3. Harpe with a blunt apical process.....*G. agamemnon*
- Harpe with a conical lobe and another triangular two pronged spinuous process.....*G. Nomius*

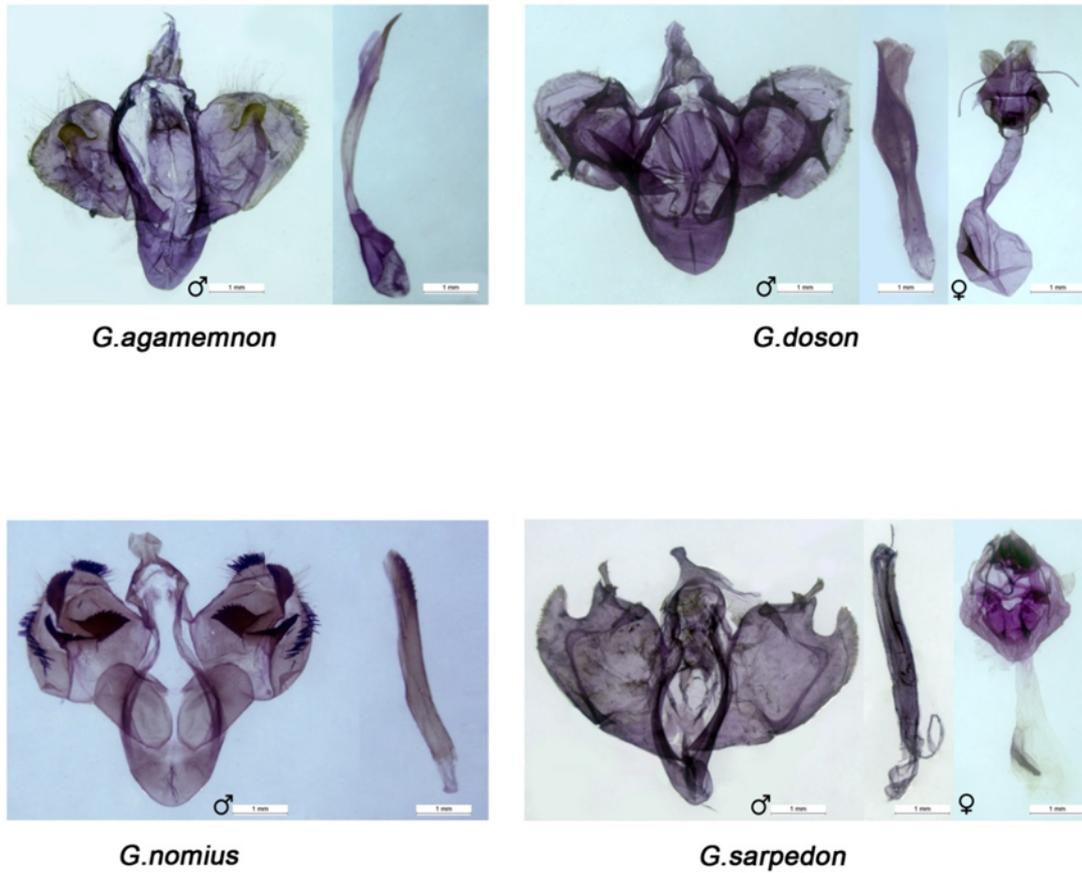


Plate 3: Photographic plates of male external genitalia. Figs: 1. *G. sarpedon*; 2. *G. doson*; 3. *G. agamemnon*; 4. *G. nomius*

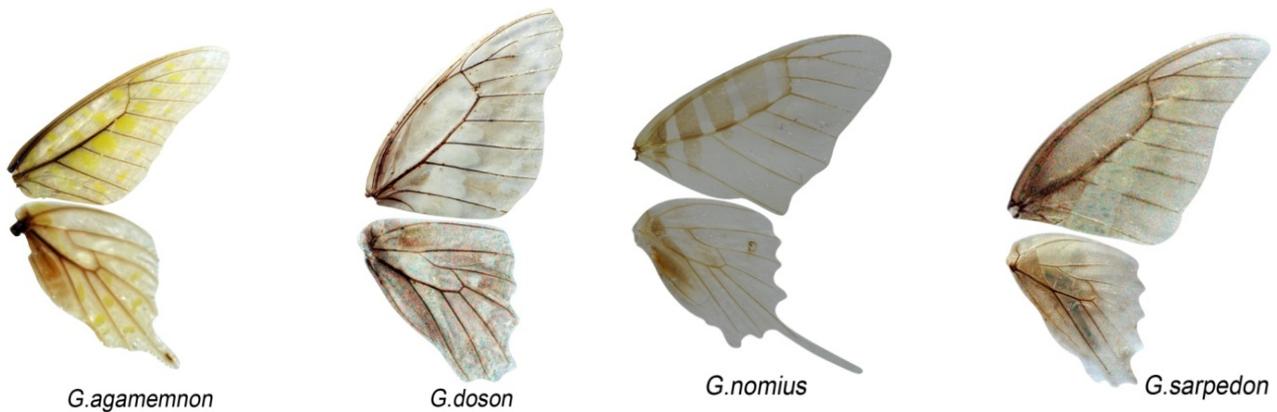


Plate 4: wing venation. Figs: 1. *G. agamemnon*; 2. *G. doson*; 3. *G. nomius*; 4. *G. sarpedon*

4. Conclusion

The structure of the various parts of the genitalia was found to be highly divergent indicating that each species that are phylogenetically distant. Morphology of uncus, valvae, saccus and phallus of the male genitalia were found to provide a satisfactory basis for taxonomic segregation. Among these, harpe was the most important structure for segregating the Papilionids recorded here. Being an economically important tribe, conservation of these species is very important and the information generated in this study will be useful in developing appropriate conservation strategies.

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